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SPLENOMEGALY AND MALARIA IN THE CENTRAL HIGHLANDS OF SOUTH VIETNAM*†

EDWARD J. COLWELL, LLEWELLYN J LEGTERS,‡ AND EARL H. FIFE, JR.
Walter Reed Army Institute of Research, Washington, D.C. 20012

ABSTRACT: Studies on the prevalence and etiology of splenomegaly were accomplished at three hamlets of a geographically remote village in South Vietnam. The percent of children and adolescents with enlarged spleen ranged from 70 to 90. Several with Class 4 or 5 spleen enlargement were observed. Single or mixed malaria infections were demonstrated in 19 of 42 splenomegalic children. In contrast to previous highland surveys, *Plasmodium malariae* infections were found in five. Thirty-three of these children, chosen without regard to spleen size, were selected for further study at base camp. Microhematocrit values ranged from 30 to 44%, and five of the 33 children had anemia secondary to iron deficiency. Some of the nonanemic children exhibited hematologic abnormalities that included target cells and bone-marrow erythrohyperplasia. Parasitologic studies for leishmaniasis were negative, although five of 25 children had positive fluorescent-antibody tests for *Leishmania donovani* antibody. Comparison of malaria complement-fixation (CF) tests and blood-film examinations were made in 16 children. Fositive reactions were demonstrated in 15, whereas only seven had demonstrable parasitemia. Moreover, the CF tests indicated mixed *Plasmodium* infections in 11 children, compared with only two mixed infections demonstrated by blood-film examinations. The results indicated that malaria infections were the primary cause for splenomegaly in those examined, although the beta-thalassemia trait was not excluded as a contributory factor.

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Splenomegaly is a common physical finding in residents of the Central Highlands of South Vietnam. The rural areas of this mountainous terrain are inhabited primarily by people whom the French termed *Montagnards* (meaning "hill people"). It is generally believed that their ancestors were of ancient Cambodian and Malayo-Polynesian origin, who migrated to the Indochina peninsula about 3,000 to 5,000 years ago.[1] Splenic enlargement among these tribespeople has generally been attributed to malaria, which is hyperendemic in the Highlands.[2] However, surveys for other causes of splenomegaly among this ethnic group have not been reported.

This report describes studies on the possible etiologies of splenomegaly in residents of a geographically remote village in the Central Highlands.

MATERIALS AND METHODS

The site selected for study was a village called Gia Vuc, which is located about 80 km south of Da Nang and 40 km inland from the South China Sea. The village consists of three hamlets of recent construction, to accommodate an influx of refugees who came from as far away as 15 km during the past 4 years. The total population numbered about 2,500 of which 80% were Montagnards of Hre (Malayo-Polynesian) tribal origin and the remainder of Vietnamese extraction.

Examinations for splenomegaly were accomplished on several hundred ambulant children and adolescents in each hamlet. Age and spleen size were not recorded for most of those examined. Because of military and farming duties, most men and women were not available for examination.

Single thick and thin blood films were obtained from 42 splenomegalic children, ranging in age from 5 to 14 years. Thirty-three of these children, chosen without regard to spleen size, were brought to base camp where additional studies were accomplished. Physical examinations were performed, and oral temperatures and abnormalities

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† In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

‡ Present address: Division of Preventive Medicine, U. S. Army Hospital, Fort Ord, California 93941

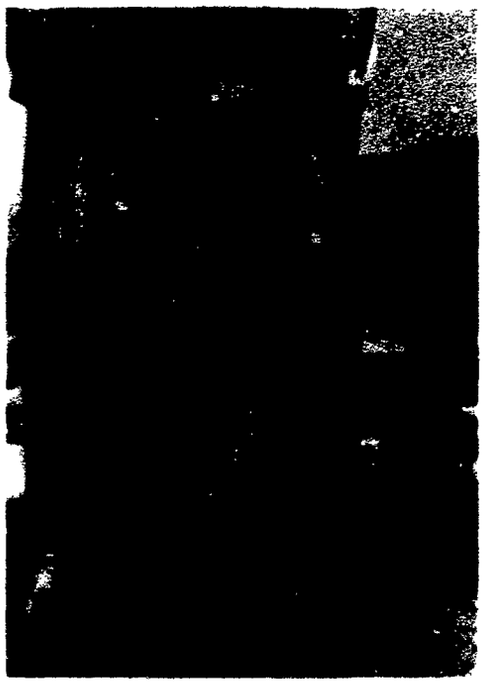

FIGURE 1. Photograph showing Class 5 splenomegaly in a Montagnard child from Gia Vuc.

of the liver, spleen, and lymph nodes were recorded. Specimens of peripheral blood were obtained for a complete blood count, serum bilirubin, albumin, and globulin, and certain serologic tests. The latter included the formol-gel test,[3] the fluorescent-antibody (FA) test for *Leishmania donovani*,[4] and the complement-fixation (CF) test for *Plasmodium* infections.[5] In the CF test for malaria, purified, soluble antigens of *P. falciparum*, *P. knowlesi*, and *P. malariae*, obtained from experimentally infected sub-human primates, were utilized.[6, 7]

A bone-marrow aspiration was accomplished aseptically in all children studied at the base camp. Wright's, Giemsa, and Prussian blue iron stained preparations were examined for morphologic abnormalities, sideroblasts, and protozoan parasites. Aspirates from the first 22 children were inoculated intraperitoneally into paired hamsters. The animals were killed 16 to 24 weeks later, and stained splenic imprints were examined for *Leishmania*.

Stool specimens were obtained from 12 of the children and examined for eggs and parasites.[8] In addition, the lead content of several random urine specimens and of samples from the local water source and cooking utensils was determined.[9] Blood specimens from 12 adult Hre tribesmen were obtained for hemoglobin typing by starch-gel electrophoresis.[10]

TABLE 1

Results of single blood film examinations for Plasmodium *infections in 42 children with splenomegaly*

Species	Positive (No.)	Positive (%)
P. falciparum	13	30.0
P. vivax	7	16.7
P. malariae	5	11.9
Not identified	1	2.4

RESULTS

Rates of splenomegaly among children and adolescents in the three hamlets ranged from 70 to 90%. Several children with class 4 and 5 splenic enlargement (according to Hackett[11]) were observed; however, age-specific spleen size could not be recorded. A child from this village with massive splenomegaly is shown in Figure 1.

Single or mixed malaria infections were demonstrated in 19 of 42 splenomegalic children from whom single blood films were obtained in the field. Frequencies of the respective *Plasmodium* infections are shown in Table 1. The observed frequencies demonstrated that this area is highly endemic for malaria. Moreover, in contrast to a previous survey,[2] *P. malariae* infections were detected in some of these children.

Spleen sizes in the 33 children examined at base camp ranged from class 1 to 3 in all but one, who had class 4 enlargement. The frequencies of clinical and hematologic abnormalities among this group are shown in Table 2. Oral temperatures exceeding 100° F were demonstrated in 28 of 33 children. Four of the children had generalized lymphadenopathy, and one of these had moderate hepatomegaly associated with class 4 splenomegaly.

Microhematocrits ranged from 30 to 44%. According to the normal microhematocrits for this age group,[12] five of the 33 had anemia. Examination of the peripheral thin-blood smears from the anemic children revealed mild to moderate degrees of hypochromia, microcytosis, and basophilic stippling of erythrocytes. Examination of their stained marrow-aspirate preparations

TABLE 2

Frequency of clinical and hematologic abnormalities in 33 splenomegalic children

	Positive	
	(*No.*)	(%)
Fever	28	84.8
Hepatomegaly	10	30.3
Generalized adenopathy	4	12.1
Eosinophilia	19	57.6
Target cells	9	27.3
Anemia	5	15.2
Leukocytosis	3	9.1
Leukopenia	1	3.0
Marrow erythroid hyperplasia	13	39.4
Marrow iron depletion	10	30.3

showed erythrohyperplasia with inadequate hemoglobinization of normoblasts and decreased uptake of Prussian blue iron stain. In two of the anemic children, target cells constituted 3% and 40% of the red-cell populations. Hemoglobin-typing and serum-iron indices were not accomplished.

Although the remaining 28 children were nonanemic, according to Wintrobe's criteria,[12] other hematologic abnormalities were noted. In seven children, targeting of erythrocytes ranged from 2% to 18% of the red blood cells. Hyperplasia of bone-marrow erythroid elements was observed in eight children, and in five of these, bone marrow aspirates showed a decreased uptake of Prussian blue iron stain.

Blood leukocyte counts ranged from 3,750 to 19,500 per cmm. However, only three of 33 were considered to have leukocytosis (*i.e.*, over 15,000 per cmm at ages 5 to 10 years[13]). Absolute hypereosinophilia was observed in 27 children, and most showed eosinophilic hyperplasia of bone-marrow myeloid elements. Single stool examinations were accomplished in 11 children, and 10 were found to be infected with hookworm or *Ascaris lumbricoides*, or both.

Because of the frequent observation of erythrocytic basophilic stippling, the possibility of chronic lead intoxication was considered. However, the lead content of random urine specimens from two children and specimens from the water source and cooking utensils were within normal ranges. Starch-gel electrophoresis of blood specimens from 12 adult Montagnard tribesmen from Gia Vuc revealed the hemoglobin E trait in one person.

Serum specimens for biochemical and serologic tests were obtained from 28 of 33 persons. None had hyperbilirubinemia, and only three exhibited depressed levels of serum albumin (*i.e.*, below 3 g per 100 ml). However, hyperglobulinemia (*i.e.*, over 3.5 g per 100 ml) was demonstrated in 27 children. Fifteen of the latter had serum globulin levels over 5.0 g per 100 ml. Five of 25 children had positive FA tests for *L. donovani* infection. However, all formol-gel tests were negative, *Leishmania* could not be demonstrated in Giemsa-stained preparations of bone-marrow aspirates, and splenic imprints from the marrow-inoculated hamsters were also negative.

TABLE 3

Comparison of parasitologic findings, spleen size, and malaria CF tests in 16 children

		Reactivity with indicated antigen in CF tests*		
Blood film	Spleen size	PF	PK	PM
PF, PM	2	1	W†	0
PF, PV	1	0	W	W
PF	2	>128	4	0
PF	4	>128	64	1
PF	2	16	W	1
PV	2	64	W	0
P	1	4	1	0
0	3	>128	4	W
0	2	>128	0	4
0	3	>128	4	0
0	3	>128	4	0
0	1	>128	0	1
0	2	4	0	0
0	2	1	0	W
0	3	R(AC)†	R(AC)	0
0	3	R(AC)	R(AC)	1

* PF, *P. falciparum* antigen; PK, *P. knowlesi* antigen (for detection of *P. vivax* antibody); and PM, *P. malariae* antigen.
† W indicates weak reaction, and R(AC) is reactive but anticomplementary; the figures represent serum titers.

Serum specimens from 16 children were examined in CF tests with the three *Plasmodium* antigens. Table 3 summarizes the results of the CF tests in comparison with spleen size and peripheral blood-film examinations. Significant titers (*i.e.*, one or greater) for malaria CF antibodies were detected in 15 children, but only seven had patent parasitemia. Although *P. malariae* parasites could be demonstrated in only one child, five showed significant titers with purified *P. malariae* antigen. Furthermore, the CF tests indicated the presence of mixed infections in 11 children, whereas only two were shown to have mixed infections by direct parasitologic

examinations. Finally, children with splenomegaly greater than class 2 generally showed positive CF tests with more than one antigen; whereas those with class 1 or 2 enlargement demonstrated serореactivity with only a single antigen. However, the two children with mixed infections and the one with vivax malaria showed little or no reactivity in CF tests for these infections.

DISCUSSION

Among the etiologies of splenomegaly that should be considered in this report are kala azar, liver disorders, malaria, and defective hemoglobin synthesis, particularly the thalassemia disorders. Although five of 25 children had positive FA tests for *L. donovani* antibody, falsely positive reactions with this test have been reported in serum from persons with malaria.[4] Moreover, the negative formol-gel tests and the failure to demonstrate *Leishmania* in stained marrow aspirates, and in stained splenic imprints from hamsters inoculated with marrow aspirates, virtually exclude visceral leishmaniasis as a cause of splenomegaly.

Because of the lack of local hospital facilities, closed needle biopsies of the liver could not be accomplished. However, we believe that the possibility that hepatic disorders are the primary cause for splenomegaly is unlikely. The absence of ascites, jaundice, angiomata, muscle wasting, and the low frequency of hypoalbuminemia argue strongly against chronic liver disease. Moreover, splenomegaly is an uncommon manifestation in most of the acute forms of hepatitis of infectious and toxic etiology.[14-16]

The frequent finding of a mild, microcytic anemia with target cells in these splenomegalic children raises the possibility of a genetic disturbance in hemoglobin synthesis, particularly the thalassemia trait or the homozygous hemoglobin E phenotype. Surveys for abnormal hemoglobins among four different Montagnard tribes have been accomplished; the only abnormal hemoglobin demonstrated was hemoglobin E.[17] The allele frequencies for E ranged from 3 to 37%, and the observed frequencies for the homozygous EE condition ranged from 0 to 17%. At Gia Vuc, we are reluctant to suggest a genetic disturbance of hemoglobin synthesis as the primary etiology for splenomegaly for the following reasons. First, anemic children were shown to have bone-marrow iron depletion rather than the iron overloading classically described in the thalassemias and hemoglobinopathies. It is conceivable that these anemic children could have had iron deficiency secondary to a deficient intake, or excessive loss superimposed on a genetic defect in hemoglobin synthesis. However, one would expect moderate to severe anemia if the two conditions coexisted. Again, the extremely high rates of spleen enlargement (*i.e.*, 70 to 90%) encountered in this village indicate the presence of another etiologic agent(s).

Russell has stated that he has never observed a rate of spleen enlargement greater than 5% in a nonmalarious area,[18] except in areas hyperendemic for kala azar, exanthematous diseases, or in a group recently vaccinated against smallpox. The spleen indices observed in the hamlets of Gia Vuc indicate that malaria is highly endemic and perhaps highly stable. Unfortunately, adults were not available for examination, and the military situation did not permit follow-up studies to confirm the stability of malaria endemicity.

Since World War II, quartan malaria has been reported in North Vietnamese,[19] but not in residents of South Vietnam. Recently, however, this infection has been reported in American servicemen assigned to the Central Highlands of the latter country.[20] Several factors may contribute to the failure to find *P. malariae* infections in South Vietnam. Because of the geographical and tactical circumstances in the Highlands, most malaria surveys necessarily have been limited to obtaining only a single blood film. Surveys based on single blood-film examinations can be expected to yield low frequencies of positive findings, especially in the case of *P. malariae* infections, which are characterized by low levels of parasitemia, and by prolonged latency. Furthermore, the effect of partial immunity, which can depress the level of parasitemia, is another limitation to single examinations.[21] Nevertheless, the fact that nonimmune persons have acquired quartan malaria is conclusive evidence that *P. malariae* is endemic in the area.

Recent studies in Uganda have revealed, after diligent examination of multiple peripheral blood films, parasites of *P. malariae* in about 50% of the patients with marked cryptogenic splenomegaly.[22] Subsequent serologic and histopathologic studies demonstrated that these splenomegalic patients had high titers in FA tests with *P. falciparum* antigens, and tissue from their livers

exhibited a peculiar abnormality.[23,24] The latter consisted of portal lymphocytic infiltrates and Kupffer's cell hyperplasia. These observations led those investigators to postulate that the splenomegaly was secondary to an immunologic disorder associated with quartan malaria. In South Vietnam, class 4 and 5 splenic enlargement is frequently observed, particularly in Montagnard children residing in the Central Highlands. Our studies did not indicate a definite association between splenomegaly and quartan malaria; however, this possibility can not be excluded, and we suggest that more detailed investigations are warranted to define the possible role of quartan malaria in the pathogenesis of cryptogenic splenomegaly in this country.

Recent innovations in methods for effectively separating *P. falciparum* and *P. vivax* from host-erythrocyte components and for purification of the serologically active antigen fractions[6,7] have eliminated many of the undesirable components present in the earlier crude preparations. This has resulted in a significant improvement in the specificity and sensitivity of these tests. Similar methods were employed in preparing *P. malariae* antigen obtained from a chimpanzee whose spleen had been removed. Although comprehensive evaluation of this malariae antigen is still in progress, preliminary findings have shown a high degree of sensitivity and specificity.

All serum specimens from chimpanzees experimentally infected with *P. malariae* reacted in CF tests with the *P. malariae* antigen. However, the maximum titers observed in these serum samples generally were lower than the titers exhibited in CF tests for vivax or falciparum malaria. Because there have been relatively few investigations involving experimental infection of volunteers with *P. malariae*, there has been little opportunity to evaluate the malariae antigen in cases in man in which intercurrent infection with other *Plasmodium* species could be excluded. Nevertheless, in view of the reactivity observed in serum from the experimentally infected non-human primates it is believed that antibodies would be readily detected in human beings with naturally acquired infections. The *P. malariae* antigen has shown little or no cross-reactivity with serum from patients with vivax or falciparum malaria.

Certain children in this study showed an unexpected suppression of immune response, evidenced by surprisingly low antibody levels during a period of patent parasitemia. Two of these were children with mixed infections (Table 3). The fact that both children with demonstrable mixed infections responded in this manner, whereas most children with apparent single infections showed high antibody titers, suggests that the dual infection may have been a contributing factor. Although other plausible explanations could be advanced, it is possible that this could be analogous to the phenomenon observed in concurrent, mixed-virus immunization[25] in which there is a competitive inhibition of immunologically competent cells, reducing the antibody response to multiple antigenic stimuli. In any event, it was apparent from these studies that in a malarious community in which the effects of partial immunity may suppress the clinical manifestations and degree of parasitemia, the serologic procedure provides a more realistic appraisal of the malaria experience of the group than does examination of single blood films.

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